

Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) **EP 0 773 296 A1**

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
14.05.1997 Bulletin 1997/20

(21) Application number: 96901509.8

(22) Date of filing: 01.02.1996

(51) Int. Cl.⁶: **C12N 15/81**, C12N 15/31,
C12N 1/19, C12P 21/02
// (C12N1/19, C12R1:645)

(86) International application number:
PCT/JP96/00198

(87) International publication number:
WO 96/23890 (08.08.1996 Gazette 1996/36)

(84) Designated Contracting States:
DE FR GB

(30) Priority: 03.02.1995 JP 17167/95

(71) Applicant: ASAHI GLASS COMPANY LTD.
Chiyoda-ku Tokyo 100 (JP)

(72) Inventors:
• HAMA, Yuko
Asahi Glass Company Ltd.
Yokohama-shi Kanagawa 221 (JP)
• TOHDA, Hideki
Asahi Glass Company Ltd.
Yokohama-shi Kanagawa 221 (JP)
• TSUKAMOTO, Hiroko
Asahi Glass Company Ltd.
Yokohama-shi Kanagawa 221 (JP)

• NIKAIDO, Kiyokazu
Asahi Glass Company Ltd.
Yokohama-shi Kanagawa 221 (JP)
• KUMAGAI, Hiromichi
Asahi Glass Company Ltd.
Kanagawa-ku, Yokohama-shi Kanagawa 221
(JP)

(74) Representative: Wächtershäuser, Günter, Prof.
Dr.
Patentanwalt,
Tal 29
80331 München (DE)

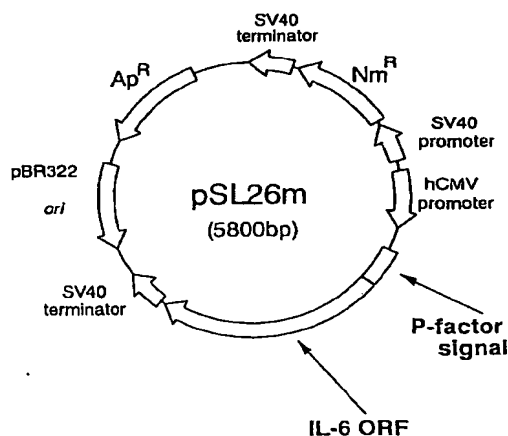
Remarks:

The applicant has subsequently filed a sequence
listing and declared, that it includes no new matter.
(0450).

(54) **SECRETORY SIGNAL GENE AND EXPRESSION VECTOR HAVING THE SAME**

(57) A secretion signal gene having a base sequence encoding a polypeptide functional as a secretion signal in fission yeast *Schizosaccharomyces pombe* (hereinafter referred to as *S.pombe*), an expression vector having the gene and a foreign protein structural gene, a multicloning vector for construction of the expression vector which has the secretion signal gene, and efficient secretory production of the foreign protein structural gene product by *S.pombe* and the like.

FIGURE 1



EP 0 773 296 A1

Description

TECHNICAL FIELD

5 The present invention relates to a secretion signal gene having a base sequence encoding a polypeptide derived from the secretion signal of a precursor of a mating pheromone (P-factor), which concerns mating of fission yeast Schizosaccharomyces pombe (hereinafter referred to as S.pombe). The present invention also relates to an expression vector containing the secretion signal gene and a foreign protein structural gene, and efficient secretory production of the foreign protein structural gene product by S.pombe or the like.

BACKGROUND ART

Hitherto, production of foreign proteins utilizing genetic recombination technology has been extensively conducted by using microorganisms such as Escherichia coli, Saccharomyces cerevisiae or Bacillus, animal cells, plant cells and insect cells. As such foreign proteins, various biogenic proteins are considered to be accessible, and many of them have been industrially produced by using these living organisms for medical use so far.

However, methods employing procaryotes are not effective for all polypeptides, and it is not always easy to reproduce the complicated post-translational modification of eucaryotic proteins and to reproduce the natural steric structures. In addition, Escherichia coli has a characteristic endotoxin, which might contaminate end products. On the other hand, as for methods employing animal, plant or insect cells, these cells are more difficult to handle than microorganisms, their culture is costly, and production efficiency is low. For this reason, yeasts, eucaryotic microorganisms, are considered as the best for production of foreign proteins, especially eucaryotic proteins. Their culture methods are well established, and they do not contain endotoxins. Therefore, expression vectors for use in various yeast hosts have been developed so far (Romanos, M.A. et al., Yeast 8, 423-488, 1992).

Among various yeasts, S.pombe is considered to be closer to higher animals in various properties such as cell cycle, chromosomal structure and RNA splicing than other yeasts inclusive of Saccharomyces cerevisiae. The post-translational modification such as acetylation, phosphorylation and glycosylation of proteins produced in S.pombe seems fairly similar to that in animal cells (Russell, P.R. and Nurse, P., Cell 45, 781-782, 1986; Kaufer, N.F. et al, Nature 318, 78-80, 1985; Chappell, T.G. and Warren, G., J. Cell. Biol. 109, 2693-2702, 1989). Therefore, use of S.pombe as a host for expression of a foreign protein is expected to provide a gene product closer to its natural form, like that produced by animal cells. Since yeasts have a lot of commonness in their culture methods, knowledges about other yeasts can be easily applied to the yeast. Therefore, it is obviously advantageous to use S.pombe for production of a foreign protein by using microbiological methods and the DNA recombination technique.

However, S.pombe is far behind Escherichia coli and Saccharomyces cerevisiae in studies on genetic recombination using them. Especially, with respect to gene expression in S.pombe, only a small number of studies have been reported (Japanese Unexamined Patent Publications Nos. 181397/1986, 283288/1990 and 63596/1992). This is because development of expression vectors which have powerful promoters, are stable in S.pombe cells and are suitable and convenient for introduction of a gene has been retarded. Recent development of vectors for the fission yeast with a high expressivity which contains an animal virus-derived promoter region eventually opened the way to mass production of foreign proteins by S.pombe (Japanese Unexamined Patent Publications Nos. 15380/1993 and 163373/1995, which disclose inventions of the present inventors). This technique enabled many intracellular proteins to be produced easily and therefore is fairly useful.

Production of (foreign) eucaryotic secretory proteins by yeasts scarcely succeeded so far because yeasts can hardly recognize inherent signal sequences of foreign secretory proteins and therefore can not secrete the products from the cells into culture media. Further, at the time of purification, it was necessary that after cell rupture, the desired protein should be isolated from various coexistent cell components to avoid inactivation. Secretory production of a foreign protein is not only preferable in view of the easiness of purification, but also advantageous in that the product is identical or fairly similar to its naturally occurring counterpart in steric structure, because the protein to be secreted enters the secretory pathway in the host cells and undergoes appropriate processings such as formation of disulfide bonds and glycosylation.

However, few signal sequences that effectively function in the fission yeast have been reported (Tokunaga, M. et al., Yeast 9, 379-387, 1993; Bröcker, M. et al., B.B.A. 908, 203-213, 1987), no secretory expression vectors have been practically developed. On the other hand, the present inventors studied P-factor, which is a protein secreted by S.pombe from the cells and involved in mating as a mating pheromone. As a result, they found the fact that after conversion from its precursor by various enzymes in S.pombe, P-factor is secreted into a culture medium. They also determined the amino acid sequence and the gene of the P-factor precursor (Imai, Y. and Yamamoto, M., Gene & Dev. 8, 328-338; Japanese Unexamined Patent Publication No. 327481/1994). The amino acid sequence of the P-factor precursor is SEQ ID NO: 1 in the Sequence Listing give afterwards.

DISCLOSURE OF INVENTION

The present inventors focused on the above-mentioned P-factor and tried to develop a secretory expression vector by utilizing its secretory property. Though, it had been unknown which regions of the precursor function effectively as secretion signals as a result of further studies the present inventors found the N-terminal about 60 amino acids of the P-factor precursor functions effectively as secretion signals. As a result of more detail studies, they found the existence of a presequence which is to be cut off by signal peptidase, and a prosequence, which is to be cut off by proteases in the endoplasmic reticulum or the Golgi apparatus during processing. Accordingly, the secretion signal of the P-factor precursor can be shorter than the N-terminal about 60 amino acids.

The present invention provides a gene encoding for a polypeptide functional as a secretion signal in S.pombe. The polypeptide may be a polypeptide identical, equivalent or analogous to the secretion signal of the P-factor precursor or a longer polypeptide containing the polypeptide (but shorter than the P-factor precursor itself). The polypeptide may have at least one extra amino acid residue that the secretion signal of the P-factor precursor does not inherently have, or have at least one different amino acid residues substituted for at least one amino acid residue in the secretion signal of the P-factor precursor. Further, the polypeptide may lack at least one amino acid residue in the secretion signal of the P-factor precursor. Hereinafter, a polypeptide functional as a secretion signal in S.pombe is referred to simply as the secretion signal.

The present invention relates to a gene encoding the secretion signal (hereinafter referred to as secretion signal gene), a multicloning vector having the secretion signal gene, an expression vector having the secretion signal gene and a foreign protein structural gene, a transformant from a eucaryotic host which carries a recombinant DNA having the secretion signal gene or the expression vector, and a method of producing a foreign protein by using the transformant. The present invention provides:

a secretion signal gene having a base sequence encoding a polypeptide functional as a secretion signal in S.pombe;

a secretion signal gene having a base sequence encoding a secretion signal of the P-factor precursor produced by S.pombe or a polypeptide containing the amino acid sequence of the secretion signal and functional as a secretion signal in S.pombe, in which one or more addition, deletion or substitution of at least amino acid residue have been made;

a secretion signal gene having a base sequence encoding a polypeptide having an amino acid sequence from the 1st amino acid to the 16 - 160th amino acid of SEQ ID NO: 1, wherein addition, deletion or substitution of at least amino acid residue may have been made;

a secretion signal gene having a base sequence encoding a polypeptide having an amino acid sequence from the 1st amino acid to the 22±6th, the 31±6th or the 57±6th amino acid of SEQ ID NO: 1, wherein addition, deletion or substitution of at least amino acid residue may have been made;

a secretion signal gene having a base sequence encoding the above polypeptide, wherein the polypeptide has at least one additional amino acid residue at the carboxy terminus so as to have a carboxy-terminal amino acid sequence of [-Lys-Lys-Arg];

a multicloning vector for construction of an expression vector for expression of a foreign protein in a eucaryotic host cell, which has the above-mentioned secretion signal gene upstream from a foreign protein structural gene introduction site so that the secretion signal gene can be directly ligated with a foreign protein structural gene to be introduced;

an expression vector to be expressed in an eucaryotic host, which has a structural gene of a protein combination of a secretion signal and a foreign protein bonded together in this order from the amino terminus, wherein the gene of the secretion signal is the abovementioned secretion signal gene;

a transformant from a eucaryotic host cell, which carries a recombinant DNA containing a structural gene of a protein combination of the above secretion signal gene and a foreign protein bonded together in this order from the amino terminus, or the above expression vector; and

a method of producing a foreign protein, which comprises culturing the transformant so that the foreign protein is produced and accumulated in the culture, and collecting it.

In a transformant having the secretion signal gene of the present invention, a foreign protein synthesized with a secretion signal bonded is cut in the cells so as to shed the secretion signal, and is secreted from the cells. Therefore, it is possible to obtain a desired protein by isolating it from the culture medium, and to produce a desired protein effectively and easily.

According to the present inventors' studies, the longest secretion signal in Examples is a polypeptide segment of up to the 57th amino acid. However, in the present invention the secretion signal may be a longer polypeptide segment containing this polypeptide (in Example 1, a polypeptide of up to the 59th amino acid was used). In this case, a foreign protein is secreted with amino acid residues in the polypeptide segment downstream from the cleaved site (hereinafter

referred to as additional segment) bonded. The object of the present invention can be achieved, when the secreted protein is useful or at least not adverse (for example, when the additional segment can be removed afterward) even if the intended foreign protein is secreted with the additional segment bonded to it. Further, considering the sequence from the 55th amino acid to the 59th amino acid of the polypeptide segment, where the polypeptide segment is cleaved, the sequence of the first about 160 amino acid residues contains three more sequences similar to the sequence from the 55th to the 59th amino acid, and it is anticipated that the cleavage can occur also at these three sites.

However, in general, a long additional segment is likely to be adverse to the desired foreign protein. Accordingly, it is preferred that the secreted protein contains no additional segment or a short additional segment. In this sense, an expression vector preferably is so constructed as to induce expression of a foreign protein bonded to a sequence having an amino acid sequence of the first at most 160 amino acids of SEQ ID NO: 1 containing a secretion signal, more preferably to a sequence having the amino acid sequence (SEQ ID NO: 2), which corresponds to the first at most 59 amino acids of SEQ ID NO: 1. The results of Examples show that when the secretion signal has an amino acid sequence of up to the 59th amino acid, the secreted foreign protein has the 58th and 59th amino acid residues of the secretion signal at the amino terminus. Therefore, in this case, it is anticipated that a polypeptide from the 1st amino acid to the 57th amino acid of SEQ ID NO: 1 (or, of SEQ ID NO: 2) is more suitable as a secretion signal.

Cleavage occurs behind from the 55th amino acid to the 57th amino acid sequence of [-Lys-Lys-Arg-]. Therefore, it is anticipated that this sequence is a kind of cleavage signal. This suggests that cleavage also can occur behind the sequences of [-Lys-Lys-Arg-] from the 89th amino acid to the 91st amino acid, from the 123rd amino acid to the 125th amino acid and from the 157th amino acid to the 159th amino acid.

Further, a detailed study on the secretion signal aimed at use of a shorter sequence revealed that the amino acid sequence from the amino terminus to the 22nd amino acid constitutes the presequence, and the amino acid sequence from the 23rd (or the 25th) amino acid to the 31st amino acid constitutes the prosequence. Both the presequence and the prosequence can lead a desired protein bonded directly behind them to secretion. Therefore they are also useful as secretion signals, even though they are shorter than a sequence of the first about 60 amino acids from the amino terminus. On the other hand, according to a theoretical prediction, (von Heijne, G. "A new method for predicting signal sequence cleavage sites", Nucleic Acids Research, 14, 4683-4690 (1986)), the 16th [-Ala] and the 18th [-Pro] are possible rear ends of secretion signals shorter than the sequence of up to the 22nd amino acid. Therefore it is anticipated that a polypeptide having an amino acid sequence from the 1st amino acid to the 16th amino acid and a polypeptide having an amino acid sequence from the 1st amino acid to the 18th amino acid also can function as secretion signals.

A more detailed study revealed that the polypeptide segment of from the amino terminus to about the 60th amino acid is cleaved in a higher probability especially at the carboxy terminus of the 57th Arg than the presequence of up to the 22nd (or the 24th) amino acid and the prosequence of up to the 31st amino acid. Therefore, in the case of a secretion signal of a short sequence, attachment of the sequence of [-Lys-Lys-Arg-] of from the 55th amino acid to the 57th amino acid directly behind the presequence or the prosequence leads to easier cleavage and is expected to efficient secretion. Actually, it is demonstrated that attachment of [-Lys-Arg] directly behind the 31st [-Lys-] improves efficiency of secretory production.

From the above, it is thought that the P-factor precursor is cleaved just behind the 22nd (or the 24th), the 31st and the 57th amino acids, and the polypeptides having the sequences of from the first amino acid to these amino acids can function as secretion signals in *S.pombe*. Further, it is anticipated that longer or shorter sequences can also function as secretion signals in *S.pombe*. Therefore, the secretion signals of the present invention are primarily identical to these secretion signals of the P-factor precursor. The secretion signals of the present invention are polypeptides obtained by adding some amino acid residues to the C-termini of these polypeptides (such as the above-mentioned polypeptides of from the 1st amino acid to the 59th amino acid and Secretion Signal P2 Sequence which will be described later in Examples) and polypeptides obtained by deleting some amino acid residues at the C-termini of these polypeptides (such as Secretion Signal P1 Sequence which will be described later in Examples). Polypeptides obtained by substituting some amino acid residues of these polypeptides with other amino acid residues (such as amino acid residues analogous to the amino acid residues to be substituted in properties) also can function as secretion signals.

More preferred secretion signals are (a) a polypeptide having a sequence from the 1st amino acid to the 22nd (or the 25th) amino acid, (b) a polypeptide having a sequence of from the 1st amino acid to the 31st amino acid, (c) a polypeptide having a sequence of from the 1st amino acid to the 57th amino acid, (d) polypeptides which have the C-termini ± 6 residues (preferably ± 3 residues) of the C-termini of the polypeptides (a) to (c), and (e) a polypeptides which correspond to polypeptide (a), (b) or (d) which has at least one additional amino acid residue so as to have the sequence of [-Lys-Lys-Arg] at the C-terminus. In the case of (e), the number of additional amino acid residues is preferably at most 5.

The secretion signal gene of the present invention is a gene encoding the above-mentioned secretion signal. This secretion signal gene means genes corresponding to upstream regions of the gene of the P-factor precursor disclosed in Japanese Unexamined Patent Publication No. 327481/1994 and its modified versions. The secretion signal gene is obtainable both from a *S.pombe* chromosome and by synthesis. The secretion signal gene is by no means restricted to the sequences disclosed in the above-mentioned publication, and may be genes having sequences encoding the amino

acid sequences described above.

A desired foreign protein is expressed through an expression system containing its structural gene. As the expression system, a eucaryotic cell transformed with an expression vector containing a foreign protein structural gene is preferred. As the eucaryotic cell, *S.pombe* is particularly preferred. In the expression system, the above-mentioned secretion signal gene is linked to the front end of the foreign protein structural gene, and a protein combination of the secretion signal and the foreign protein bonded together is produced. After intracellular processing, the foreign protein is secreted out of the cell.

The multicloning vector of the present invention is a multicloning vector for construction of the expression vector which will be described below and enables expression of a foreign protein in a eucaryotic host cell. By introducing a foreign protein structural gene into the multicloning vector, the expression vector described below can be constructed. The expression vector described below means those constructed by using the secretion signal-foreign protein gene described below, and is not restricted to those constructed by using the multicloning vector of the present invention.

The multicloning vector of the present invention has the secretion signal gene described above upstream from a foreign protein structural gene introduction site so that the secretion signal gene can be ligated directly with a foreign protein structural gene to be introduced. When a foreign protein structural gene is introduced into it to constitute an expression vector, the secretion signal gene and the foreign protein structural gene are ligated and its expression of the ligation product produces a protein combination of the secretion signal and the foreign protein bonded together.

The expression vector of the present invention has a gene corresponding to the above-mentioned secretion signal and a foreign protein structural gene ligated together (hereinafter referred to as secretion signal-foreign protein gene) and enables expression of the gene in a eucaryotic host cell. This expression vector can be constructed by various methods by using the secretion signal-foreign protein structural gene. For example, it can be constructed by introducing a secretion signal-foreign protein gene at the multicloning site of a multicloning vector other than the multicloning vector of the present invention. It can be constructed without using a multicloning vector.

The expression vector of the present invention is preferably an expression vector having the structure disclosed in the above-mentioned Japanese Unexamined Patent Publication No. 15380/1993. It is preferred to construct the expression vector of the present invention by using the above-mentioned multicloning vector, which is disclosed in the specification of Japanese Patent Application No. 241581/1994, which relates to this expression vector or the method of constructing an expression vector using the multicloning vector. Nevertheless, the expression vector of the present invention may be an expression vector other than the expression vector disclosed in the above-mentioned publication, and may be of a chromosome integration type or of a type that can increase the copy number and can be present stably outside the nucleus. The expression vector of the present invention which has the structure disclosed in Japanese Unexamined Patent Publication No. 15380/1993 will be described below. However, the expression vector of the present invention is by no means restricted to it.

The expression vector of the present invention has a promoter region which controls the expression of the secretion signal-foreign protein gene introduced. The promoter governs the expression of the secretion signal-foreign protein gene introduced downstream. The promoter is capable of functioning in a eucaryotic cell and accelerates transcription of the introduced secretion signal-foreign protein gene. Specifically, a promoter which can function in *S.pombe* cells is preferred.

As such a promoter, for example, alcohol dehydrogenase gene promoter, human cytomegalovirus gene promoter and human chorionic gonadotropin α gene promoter may be mentioned. Particularly preferred are promoters which strongly accelerate transcription, such as promoters from animal viruses (R. Toyama et al., FEBS Lett, 268, 217-221 (1990)). As such a preferable promoter, promoters from animal viruses, particularly human cytomegalovirus gene promoter may be mentioned.

The expression vector of the present invention may have a drug resistance gene such as an antibiotic resistance gene and other various genes. Further, it is also possible to make the vector of the present invention a shuttle vector by incorporating a promoter or a drug resistance gene capable of functioning in a procaryotic cell such as *E. coli*.

An expression vector must have a replication origin in order to be expressed in cells. However, for the expression vector of the present invention, it is not always necessary to have a replication origin. A replication origin can be introduced after the expression vector has been constructed. It is also possible to autonomously introduce a replication origin into an expression vector having no replication origin in a cell after the expression vector is taken up by the cell. These methods of introducing a replication origin are already known. For example, a vector having a replication origin capable of functioning in a yeast (hereinafter referred to as a yeast vector) can be integrated with the expression vector of the present invention (Japanese Unexamined Patent Publication No. 15380/1993). It is also possible to let the expression vector of the present invention and a yeast vector fuse together in cells autonomously by introducing them into the same cells. Since these method of introducing a replication origin are available, it is not critical whether the expression vector of the present invention has a replication origin or not. However, in any case for expression of the expression vector in cells, it is necessary that the vector ultimately has a replication origin.

It is usually essential to introduce a drug resistance gene such as an antibiotic resistance gene into an expression vector as a marker or for cloning. A gene which releases a leucine-requiring cell from the necessity for leucine (such as

LEU2 gene) or a gene which releases a uracil-requiring cell from the necessity for uracil (such as URA3 gene) is often introduced. It is preferred also for the vector of the present invention to have an antibiotic resistance gene and a promoter which accelerates the transcription of the antibiotic resistance gene (hereinafter referred to as a second promoter). The second promoter is preferred to have a lower transcription accelerating activity than the promoter that accelerates the transcription of the above-mentioned secretion signal-foreign protein gene. As the second promoter, promoters from animal viruses are preferred. Particularly preferred is SV40 early promoter. Although the antibiotic resistance gene governed by the second promoter may be a conventional one, particularly in the present invention, neomycin resistance gene is preferred.

In the present invention, by the use of the expression vector having an antibiotic resistance gene, it is possible to increase the expression amount of the secretion signal-foreign protein gene. For this purpose, the transcription accelerating activity of the second promoter must be lower than that of the promoter that governs the secretion signal-foreign protein gene. For the purpose of explanation, the case of culturing *S.pombe* that carries an expression vector having SV40 early promoter and neomycin resistance gene governed by the promoter is given as an example. When the *S.pombe* transformant is cultured in a medium containing G418 (neomycin), the copy number of the expression vector in a cell increases with G418 concentration in the medium. Accordingly, by increasing the G418 concentration, it is possible to increase the copy number of the expression vector in a cell and thereby increase the expression amount of the secretion signal gene-foreign protein. If the activity of the second promoter is higher than the promoter governing the secretion signal-foreign protein gene, there is no need to increase the copy number of the expression vector since a small copy number of the vector is enough to induce production of a sufficient amount of neomycin resistance protein (enzyme), and therefore it is impossible to increase the expression amount of the desired secretion signal-foreign protein.

The foreign protein as the product of a foreign protein structural gene is not particularly restricted, but is preferably a physiologically active protein of a higher animal. For example, a glycoproteins which are basically obtainable by secretory production by an animal cell and difficult to produce by using *E.coli* and proteins having complicated steric structures with many disulfide bonds, such as human serum albumin and interleukin-6, are particularly preferred.

The general technique of constructing the multicloning vector or the expression vector of the present invention is already known and disclosed, for example, in a reference, J.Sambrook et al., "Molecular Cloning 2nd ed.", Cold Spring Harbor Laboratory Press (1989). The multicloning vector and the expression vector of the present invention can be constructed by the above-mentioned method by using this conventional technique. As a strain of *S.pombe* to be used in the present invention as a host for the expression vector, ATCC 38399 (leu1-32h⁻) and ATCC 38436 (ura4-294h⁻) may, for example, be mentioned. These strains are available from American Type Culture Collection.

S.pombe can be transformed by using an expression vector by known methods, and a *S.pombe* transformant can be obtained by, for example, the lithium acetate method (K.Okazaki et al., Nucleic Acids Res., 18, 6485-6489 (1990)). The transformant is cultured in a known medium, and nutrient media such as YPD medium, minimal media such as MM medium (M.D. Rose et al., "Methods In Yeast Genetics", Cold Spring Harbor Laboratory Press (1990).) and the like may be used. The transformant is cultured usually at from 16 to 42°C, preferably at from 25 to 37°C, for from 8 to 168 hours, preferably from 48 to 96 hours. Either of shaking culture and stationary culture can be employed, and, if necessary, the culture medium may be stirred or aerated.

As methods of isolating and purifying the protein produced in the culture, known methods, such as methods utilizing difference in solubility such as salting out and precipitation with a solvent, methods utilizing difference in molecular weight such as ultrafiltration and gel electrophoresis, methods utilizing difference in electric charge such as ion-exchange chromatography, methods utilizing specific affinity such as affinity chromatography, methods utilizing difference in hydrophobicity such as reverse phase high performance liquid chromatography, and methods utilizing difference in isoelectric point such as isoelectric focusing may be mentioned.

The isolated and purified protein can be identified by conventional methods such as western blotting or assay of its activity. The structure of the purified protein can be defined by amino acid analysis, amino-terminal analysis, primary structure analysis and the like.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings in association with the item best mode for carrying out the invention, are explained below.

Figure 1 illustrates the structure of the vector pSL26m, which was constructed in Example 1. Figure 2 and Figure 3 show SDS-PAGE and western blotting patterns which demonstrate expression of human interleukin-6 and its variants obtained in Examples 2, 5, 8, 11 and 14. Figure 4 illustrates the structure of the expression vector pSL2PO6a'c1 constructed in Example 4. Figure 5 illustrates the structure of the expression vector pSL2P16a'c1 constructed in Example 7. Figure 6 illustrates the structure of the expression vector pSL2P26a'c1 constructed in Example 10. Figure 7 illustrates the structure of the expression vector pSL2P36'c1 constructed in Example 13. Figure 8 illustrates the structure of the expression vector pSL2P3M1 constructed in Example 16.

BEST MODE OF CARRYING OUT THE INVENTION

Now, the present invention will be described in further detail with reference to Examples. However, it should be understood that the technical scope of the present invention is by no means restricted to such specific examples.

Reference Examples 1 and 2 explain the methods of transforming and culturing a yeast employed in Examples.

REFERENCE EXAMPLE 1: Transformation of a yeast

A leucine-requiring strain, *S.pombe* leu1-32h⁻ (ATCC38399) was used as the host. Host cells were grown in animal medium until the cell number became $(0.5-1) \times 10^7$ cells/ml. The cells were collected and suspended in 0.1 M lithium acetate (pH 5) at a cell number of 1×10^9 cells/ml. Then, the cell suspension was incubated at 30°C for 60 minutes. 1 µg of a PstI fragment of a yeast vector pAL7 (ars, stb, LEU) (Nucleic Acid Research 18, 6485-6489) and 2 µg of an expression vector obtained in Examples were added to 100 µl of the cell suspension, and 290 µl of 50% (W/V) PEG4000 (polyethylene glycol with a molecular weight of 4,000) was added, and they were mixed enough. Then, the mixture was incubated at 30°C for 60 minutes and at 43°C for 15 minutes and was allowed to stand at room temperature for 10 minutes. After removal of PEG4000 by centrifugation, the cells were suspended in an appropriate amount of a medium, and the suspension was spread on a minimum medium. The transformation ratio was at least 10^5 µg (pAL7).

An appropriate number of the transformants obtained above were harvested, and each transformant was suspended in 300 µl of water. A 3 µl portion of the suspension was spread on YEA medium (yeast extract 5g, glucose 30 g, agar 20 g/l containing G418 (25 µg/ml) and three days after, the colonies formed were picked up for use.

REFERENCE EXAMPLE 2: Culture of yeast

The transformed *S.pombe* (leu1-32h⁻) strain was cultured in 5 µl of MM medium containing 1 wt% Casamino acid and 2 wt% glucose (Alfa et al. "Experiments with Fission Yeast" Cold Spring Harbor Laboratory Press 1993) in the presence of G418 (25 µg/ml) at 32°C overnight, and 5×10^7 cells withdrawn from the culture medium were added to 50 ml of MM medium containing G418 (200 µg/ml), 1 wt% Casamino acid and 2 wt% glucose and cultured at 32°C for 48 hours. Then it was centrifuged to collect the culture medium.

EXAMPLE 1

Preparation of human interleukin-6 secretory vector

PCR was performed by using a plasmid pAG9-8-I (Japanese Unexamined Patent Publication No. 224097/1995) obtained by introducing the whole cDNA of human interleukin-6 into a commercially available vector pUC19 (sold by Boehringer Co., Ltd.) as the template and oligo DNAs shown in SEQ ID NOs:3 and 4 as primers to multiply the region containing the ORF (open reading frame) of mature interleukin-6. The fragment thus obtained was subjected to double digestion by restriction enzymes EcoRI (sold by Takara Shuzo Co.) and HindIII (sold by Takara Shuzo Co.) for terminal treatment. After agarose gel electrophoresis, the band corresponding to about 600 base pairs was excised, and a gene fragment insertion was isolated by the glass beads method by using DNA-PREP (sold by Asahi Glass Company Ltd.).

PCR was performed by using a plasmid pADMP2 (Japanese Unexamined Patent Publication No. 327481/1994) containing the whole map2 gene of *S.pombe* as the template, and oligo DNAs shown in SEQ ID NOs: 5 and 6 as primers to multiply the region containing P-factor secretion signal sequence. Double digestion by restriction enzymes BspHI (sold by New England Biolab Co.) and EcoRI for terminal treatment was followed acrylamide gel electrophoresis. The band corresponding to about 200 base pairs was excised, and a signal insertion fragment was eluted from the gel.

An expression vector pTL2M for *S.pombe* (Japanese Unexamined Patent Publication No. 163373/1995) constructed by using a vector pcD4B disclosed in Japanese Unexamined Patent Publication No. 15380/1993 was subjected to double digestion by restriction enzymes AflIII (sold by New England Biolab Co.) and HindIII, and then to agarose gel electrophoresis. The band corresponding to about 5,000 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

These three fragments were ligated by means of a DNA ligation kit (Takara Shuzo Co.). *E.coli* DH5 strain (sold by Toyobo Co.) was transformed and screened for possession of the intended plasmid constructed properly. Figure 1 illustrates the structure of the expression vector pSL26m thus obtained. pSL26m was prepared in a large amount by the alkali SDS method and identified by restriction map and partial base sequence analyzes as a plasmid having the intended sequence. The amino acid sequence of human interleukin-6 anticipated from the base sequence was SEQ ID NO: 7 and consisted of 185 residues. The amino acid sequence of the secretion signal sequence was SEQ ID NO: 2 and consisted of 59 residues.

EXAMPLE 2

Secretory production of human interleukin-6

Yeast was transformed with the secretory vector prepared in Example 1 in accordance with Reference Example 1 and then cultured in accordance with Reference Example 2. 50 ml of the culture medium was concentrated about 200 times by means of a membrane filter manufactured by Amicon Co., Ltd. The concentrated sample was analyzed by SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue staining. Figure 2 shows the resulting SDS-PAGE pattern. In Figure 2, lane 1 is the supernatant of *S.pombe*/pSL2M (control) culture, and lane 2 is the super-

natant of *S.pombe*/pSL26m culture. As shown in Figure 2, several bands corresponding to molecular weights of at least 50,000 were observed, while in the low molecular weight region, a band at about 21 K and another band at a little smaller molecular weight were mainly detected.

The results of western blotting analysis of the supernatants were shown in Figure 3. In Figure 3, lane 1 is the supernatant of *S.pombe*/pSL2M (control) culture, cutting and lane 2 is the supernatant of *S.pombe*/pSL26m culture. The bands at 21 K and a little lower molecular weight were identified as attributed to human interleukin-6. Bands of little lower molecular weights seemed attributed to decomposition products.

EXAMPLE 3

Determination of the amino-terminal sequence of secreted protein

The protein isolated from the 21 K band obtained by the SDS-PAGE electrophoresis in Example 2 was analyzed from the amino terminus by a protein sequencer ("Shimadzu PSQ-I") and found to have an amino terminal sequence of Glu-Phe-Met-Pro-Val-Pro-Pro-. This indicates that it is secreted into the medium after accurate processing between Lys and Glu of the secretion signal. Similar investigation of the minor band of a lower molecular weight revealed an extra cleavage between the 9th Lys and the 10th Asp.

EXAMPLE 4

Preparation of secretory vector for interleukin-6 variant by using secretion signal

PCR was performed by using a plasmid pTL26a'C1 (Japanese Unexamined Patent Publication No. 224097/1995) containing the cDNA of a human interleukin-6 variant as the template and oligo DNAs represented by SEQ ID NOs: 8 and 9 to multiply a region containing the ORF of the interleukin-6 variant. The fragment thus obtained was subjected to double digestion by restriction enzymes EcoRI and HindIII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to about 600 base pairs was excised, and a gene insertion fragment was isolated by the glass beads method by using DNA-PREP.

A plasmid pSL26m (Example 1) containing cDNA of P-factor secretion signal sequence of *S.pombe* was subjected to double digestion by restriction enzymes EcoRI and HindIII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to 5,000 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

The two fragments were ligated by means of a DNA ligation kit. After transformation of *E.coli* DH5 strain, the *E.coli* strain was screened for possession of the secretory vector pSL2P06a'C1 properly constructed as shown in Figure 4 through restriction map analysis. pSL2P06a'C1 was prepared in a large amount by the alkali-SDS method, and the base sequences of the ORF of the interleukin-6 variant and the region corresponding to the P-factor secretion signal sequence were determined. As a result, the amino acid sequence of the interleukin-6 variant was expected from the base sequence to be represented by SEQ ID NO: 10 and consist of 162 residues, and the secretion signal sequence, which was designated as secretion signal "P0" sequence, was made of 59 residues and had an amino acid sequence represented by SEQ ID NO: 2.

EXAMPLE 5

Secretory production of human interleukin-6 variant by using secretion signal P0 sequence.

Yeast was transformed with the secretory vector prepared in Example 4 in accordance with Reference Example 1 and then cultured in accordance with Reference Example 2. 50 ml of the culture medium was concentrated about 200 times by means of a membrane filter manufactured by Amicon Co., Ltd. The concentrated sample was analyzed by SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue staining. Figure 2 is the resulting SDS-

PAGE pattern. In Figure 2, lane 3 is the supernatant of S.pombe/pSL2P06a'C1 culture.

As shown in Figure 3, while several bands were detected in the region of molecular weight of at least 50,000, in the region of lower molecular weight, only one band was detected at about 18 K.

The results of western blotting analysis of the supernatant of the culture are shown in Figure 3. Lane 3 in Figure 3 is the supernatant of the S.pombe/pSK2P06a'C1 culture. The band at 18 K in lane 3 in Figure 3 was identified as attributed to a human interleukin-6 variant (IL-6a'C1).

EXAMPLE 6

Determination of the amino-terminal sequence of secreted protein

The protein extracted by a conventional method from the band at 18 K obtained by SDS-PAGE electrophoresis in Example 5 was analyzed from the amino terminus by a protein sequence ("Shimadzu PSQ-I") and found to have an amino terminal sequence of Glu-Phe-Pro-Val-Pro-Pro-Thr-Ser-Ser-Glu. This indicates that IL-6a'C1, which is a variant which lacks the 9th and the 10th Lys-Asp from the N terminus of interleukin-6, is secreted into the medium after accurate processing between Lys and Glu of the secretion signal. Therefore, the extra cleavage in Example 3 is unnecessary to secretion, and unless a specific sequence is present in the molecule, only a type of protein with a constant terminus is secreted.

EXAMPLE 7

Preparation of secretory vector for interleukin-6 variant by using secretion signal P1 sequence

PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the cDNA of a human interleukin-6 variant as the template and oligo DNAs represented by SEQ ID NOs: 11 and 12 to multiply a region containing the ORF of the interleukin-6 variant. The fragment thus obtained was subjected to double digestion by restriction enzymes HaeII (sold by Takara Shuzo co.) and HindIII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to about 500 base pairs was excised, and a gene insertion fragment was isolated by the glass beads method by using DNA-PREP.

PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the cDNA of the P-factor secretion signal sequence of S.pombe as the template, and oligo DNAs represented by SEQ ID NOs: 13 and 14 as primers to multiply a region containing the P-factor secretion signal sequence. The fragment thus obtained was subjected to double digestion by restriction enzymes SpeI (sold by Takara Shuzo Co.) and HaeII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to about 700 base pairs was excised, and a signal insertion fragment was isolated by the glass beads method by using DNA-PREP.

An expression vector pTL2M for S.pombe (Japanese Unexamined Patent Publication No. 163373/1995) was subjected to double digestion by restriction enzymes SpeI and HindIII for terminal treatment and subjected to agarose gel electrophoresis. The band corresponding to about 4,500 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

These three fragments were ligated by means of a DNA ligation kit. After transformation of E.coli DH5 strain, E.coli clones were screened for possession of secretory vector pSL2P16a'C1 properly constructed as shown in Figure 5 through restriction map analysis.

pSL2P16a'C1 was prepared in a large amount by the alkali-SDS method and the base sequences of the ORF of the interleukin-6 variant and the region corresponding to the P-factor secretion signal sequence were determined. From the base sequences, it is anticipated that the interleukin-6 variant has an amino acid sequence represented by SEQ ID NO: 15 and made of 163 residues, and the secretion signal sequence, which is designated as secretion signal "P1" sequence, has an amino acid sequence represented by SEQ ID NO: 16 and made of 30 residues.

EXAMPLE 8

Secretory production of human interleukin-6 variant by using secretion signal P1 sequence

Yeast was transformed with the secretory vector prepared in Example 7 in accordance with Reference Example 1 and then cultured in accordance with Reference Example 2. 50 ml of the culture medium was concentrated about 200 times by means of a membrane filter manufactured by Amicon Co., Ltd. The concentrated sample was analyzed by SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue staining. Figure 2 is the resulting SDS-PAGE pattern. In Figure 2, lane 4 is the supernatant of S.pombe/pSL2P16a'C1 culture.

As shown in Figure 2, while several bands were detected in the region of molecular weight of at least 50,000, in the region of lower molecular weight, two major bands were detected at about 19 K.

The results of the western blotting analysis of the supernatant of the culture are shown in Figure 3. In Figure 3, lane 4 is the supernatant of the *S.pombe*/pSL2P16a'C1 culture. The two bands at about 19 K in lane 4 in Figure 3 were identified as attributed to a human interleukin-6 variant IL-6a'C1.

5 EXAMPLE 9

Determination of the amino terminal sequence of the secreted protein

10 The amino terminal sequences of the proteins extracted by a conventional method from the two bands at about 19 K obtained by SDS-PAGE electrophoresis in Example 5 were analyzed by means of a protein sequencer, and found to be Asp-P^{ro}-Gly-Val-Val-Ser-Val-Ser-Ala-Pro for the upper band and Gly-Val-Val-Ser-Val-Ser-Ala-Pro-Val-Pro for the lower band. The results indicate that two cleavage sites are present, and that secretion signal P1 was cut between the 22nd Ala and the 23rd Asp from the N-terminus and between Pro and Gly, which are two residues closer to the C-terminus. It is highly probable that the secretion signal P1 sequence has two signal peptidase cleavage sites.

15 EXAMPLE 10

Preparation of interleukin-6 variant secretory vector by using secretion signal P2 sequence

20 PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the cDNA of a human interleukin-6 variant as the template and oligo DNAs represented by SEQ ID NOs: 17 and 18 as primers to multiply the region containing the ORF of the interleukin-6 variant. The fragment thus obtained was subjected double digestion by restriction enzymes HaeII and HindIII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to about 500 base pairs was excised, and a gene insertion fragment was isolated by the glass beads method by using DNA-PREP.

25 PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the cDNA of P-factor secretion signal sequence of *S.pombe* as the template and oligo DNAs represented by SEQ ID NOs: 19 and 20 as primers to multiply the region containing the P-factor secretion signal sequence. The fragment thus obtained was subjected to double digestion by restriction enzymes SpeI and HaeII for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 700 base pairs was excised, and a signal insertion fragment was isolated by the glass beads method by using DNA-PREP.

30 An expression vector pTL2M for *S.pombe* (Japanese Unexamined Patent Publication No. 163373/1995) was subjected to double digestion by restriction enzymes SpeI and HindIII for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 4,500 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

35 These three fragments were ligated by means of a DNA ligation kit. After transformation of *E.coli* DH5 strain, *E.coli* clones were screened for possession of secretory vector pSL2P26a'C1 properly constructed as shown in Figure 6 by restriction map analysis.

40 pSL2P26a'C1 was prepared in a large amount by the alkali-SDS method, and the base sequences of the ORF of the interleukin-6 variant and the region corresponding to the P-factor secretion signal sequence were determined. From the base sequences, it is anticipated that the interleukin-6 variant has an amino acid sequence represented by SEQ ID NO: 15 and the secretion signal sequence, which is designated as secretion signal "P2" sequence, has an amino acid sequence represented by SEQ ID NO: 21 made of 31 residues.

45 EXAMPLE 11

Secretory production of human interleukin-6 variant by using secretion signal P2 sequence

50 Yeast was transformed with the secretory vector prepared in Example 10 in accordance with Reference Example 1 and then cultured in accordance with Reference Example 2. 50 ml of the culture medium was concentrated about 200 times by means of a membrane filter manufactured by Amicon Co., Ltd. The concentrated sample was analyzed by SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue staining. Figure 2 shows the resulting SDS-PAGE pattern. In Figure 2, lane 5 is the supernatant of *S.pombe*/pSL2P26a'C1 culture.

55 As shown in Figure 2, while several bands were detected in the region of molecular weight of at least 50,000, in the region of lower molecular weight, two major bands and one main band were detected at about 19 K and at about 18.5 K, respectively.

The results of the western blotting analysis of the supernatant of the culture are shown in Figure 3. In Figure 3, lane 5 is the supernatant of the *S.pombe*/pSL2P26a'C1 culture. The two bands at about 19 K and the band that about 18.5 K in lane 5 in Figure 3 were identified as attributed to human interleukin-6 variant IL-6a'C1.

EXAMPLE 12

Determination of the amino terminal sequence of the secreted protein

The amino terminal sequences of the proteins extracted by a conventional method from the two bands at about 19 K obtained by SDS-PAGE electrophoresis in Example 11 were analyzed by means of a protein sequencer, and found to be Asp-PRO-Gly-Val-Val-Ser-Val-Ser-Ala-Pro for the upper band and Gly-Val-Val-Ser-Val-Ser-Ala-Pro-Val-Pro for the lower band. The results indicate that two cleavage sites are present, and that secretion signal P1 was cut between the 22nd Ala and the 23rd Asp from the N-terminus and between Pro and Gly, which are two residues closer to the C-terminus. It is highly probable that the secretion signal has two signal peptidase cleavage sites. Further, the amino terminal sequence of the peptide extracted from the band at about 18.5 K was analyzed from the N-terminus and found to be Ser-Ala-Pro-Val-Pro-Pro-Thr-Ser-Ser-Glu. This indicates that the secretion signal P2 is cut between the 31st Lys and the 32nd Ser from the N-terminus during processing before secretion.

EXAMPLE 13

Preparation of interleukin-6 variant secretory vector by using secretion signal P3 sequence

PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the cDNA of a human interleukin-6 variant as the template and oligo DNAs represented by SEQ ID NOs: 22 and 23 as primers to multiply a region containing the ORF of the interleukin-6 variant. The fragment thus obtained was subjected double digestion by restriction enzymes AflII (sold by Nippon Gene Co.) and HindIII for terminal treatment and then subjected to agarose gel electrophoresis. The band corresponding to about 500 base pairs was excised, and a gene insertion fragment was isolated by the glass beads method by using DNA-PREP.

PCR was performed by using a plasmid pSL2P06a'C1 (Example 4) containing the P-factor secretion signal sequence of *S.pombe* as the template and oligo DNAs represented by SEQ ID NOs: 24 and 25 as primers to multiply a region containing the P-factor secretion signal sequence. The fragment thus obtained was subjected to double digestion by restriction enzymes SpeI and AflII for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 700 base pairs was excised, and a signal insertion fragment was isolated by the glass beads method by using DNA-PREP.

An expression vector pTL2M for *S.pombe* (Japanese Unexamined Patent Publication No. 163373/1995) was subjected to double digestion by restriction enzymes SpeI and HindIII for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 4,500 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

These three fragments were ligated by means of a DNA ligation kit. After transformation of *E.coli* DH5 strain, *E.coli* clones were screened for possession of secretory vector pSL2P36a'C1 properly constructed as shown in Figure 6 through restriction map analysis.

pSL2P36a'C1 was prepared in a large amount by the alkali-SDS method, and the base sequences of the ORF of the interleukin-6 variant and the region corresponding to the P-factor secretion signal sequence were determined. From the base sequences, it is anticipated that the interleukin-6 variant has an amino acid sequence represented by SEQ ID NO: 15 and the secretion signal sequence, which is designated as secretion signal "P3" sequence, has an amino acid sequence represented by SEQ ID NO: 26 made of 34 residues.

EXAMPLE 14

Secretory production of human interleukin-6 variant by using secretion signal P3 sequence

Yeast was transformed with the secretory vector prepared in Example 13 in accordance with Reference Example 1 and then cultured in accordance with Reference Example 2. 50 ml of the culture medium was concentrated about 200 times by means of a membrane filter manufactured by Amicon Co., Ltd. The concentrated sample was analyzed by SDS-polyacrylamide gel electrophoresis followed by the Coomassie Brilliant Blue staining. Figure 2 is the resulting SDS-PAGE pattern. In Figure 2, lane 6 is the supernatant of *S.pombe*/pSL2P36a'C1 culture.

As shown in Figure 2, while several bands were detected in the region of molecular weight of at least 50,000, in the region of lower molecular weight, one band was detected at about 18 K.

The results of the western blotting analysis of the supernatant of the culture are shown in Figure 3. The band at about 18 K in lane 6 in Figure 3 was identified as attributed to IL-6a'C1.

EXAMPLE 15

Determination of the amino-terminal sequence of the secreted protein

The N-terminal sequence of the protein isolated by a conventional method from the band at 20 K obtained by the SDS-PAGE electrophoresis in Example 14 was analyzed by a protein sequencer and found to be Ala-Pro-Val-Pro-Pro-Thr-Ser-Ser-Glu-. This indicates that the protein is secreted into the medium after accurate processing between Lys at the terminus of P3 signal and Ala at the N-terminus of LI-6a'C1.

EXAMPLE 16

Preparation of general-purpose secretory vector

PCR was performed by using an expression vector pTL2M for S.pombe (Japanese Unexamined Patent Publication No. 163373/1995) as the template and oligo DNAs represented by the SEQ ID NOs: 27 and 28 as primers to multiply the region containing a MCS (multicloning site) sequence. The fragment thus obtained was subjected to double digestion by restriction enzymes AflIII and BglII (sold by Takara Shuzo Co.) for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 300 base pairs was excised, and a MCS insertion fragment was isolated by the glass beads method by using DNA-PREP.

A human interleukin-6 variant secretory vector pSL2P36a'C1 (Example 13) was subjected to double digestion by restriction enzymes AflIII and BamHI (sold by Takara Shuzo Co.) for terminal treatment and then to agarose gel electrophoresis. The band corresponding to about 4,500 base pairs was excised, and a vector fragment was isolated by the glass beads method by using DNA-PREP.

These two fragments were ligated by means of a DNA ligation kit. After transformation of E.coli DH5 strain, E.coli clones were screened for possession of a secretory vector pSL2P3M1 constructed properly as shown in Figure 8.

pSL2P3M1 was prepared in a large amount by the alkali-SDS method, and the base sequences of the MCS sequence and the region corresponding to the P-factor secretion signal sequence were determined. As a result, the MCS sequence has a base sequence of 75 bp represented by SEQ ID NO: 29, and it is anticipated from the base sequence that the amino acid sequence of the secretion signal sequence is represented by the SEQ ID NO: 26.

Sequence Listing

SEQ ID NO: 1

SEQUENCE LENGTH: 201 amino acids

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: protein

Sequence

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala

1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Lys Ser

20 25 30

Tyr Ala Asp Phe Leu Arg Val Tyr Gln Ser Trp Asn Thr Phe Ala Asn

35 45

Pro Asp Arg Pro Asn Leu Lys Lys Arg Glu Phe Glu Ala Ala Pro Ala

50 55 60

Lys Thr Tyr Ala Asp Phe Leu Arg Ala Tyr Gln Ser Trp Asn Thr Phe

65 70 75 80

Val Asn Pro Asp Arg Pro Asn Leu Lys Lys Arg Glu Phe Glu Ala Ala

81 85 90 95

Pro Glu Lys Ser Tyr Ala Asp Phe Leu Arg Ala Tyr His Ser Trp Asn

100 105 110

Thr Phe Val Asn Pro Asp Arg Pro Asn Leu Lys Lys Arg Glu Phe Glu

115 120 125

Ala Ala Pro Ala Lys Thr Tyr Ala Asp Phe Leu Arg Ala Tyr Gln Ser

130 135 140

Trp Asn Thr Phe Val Asn Pro Asp Arg Pro Asn Leu Lys Lys Arg Thr

145 150 155 160

EP 0 773 296 A1

Glu Glu Asp Glu Glu Asn Glu Glu Glu Asp Glu Glu Tyr Tyr Arg Phe
165 170 175

Leu Gln Phe Tyr Ile Met Thr Val Pro Glu Asn Ser Thr Ile Thr Asp
180 185 190

Val Asn Ile Thr Ala Lys Phe Glu Ser
195 200 201

SEQ ID NO: 2

SEQUENCE LENGTH: 59 amino acids

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: peptide

Sequence

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala
1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Lys Ser
20 25 30

Tyr Ala Asp Phe Leu Arg Val Tyr Gln Ser Trp Asn Thr Phe Ala Asn
35 40 45

Pro Asp Arg Pro Asn Leu Lys Lys Arg Glu Phe
50 55 59

SEQ ID NO: 3

SEQUENCE LENGTH: 30 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

GAATTCATGC CAGTACCCCC AGGAGAAGAT

SEQ ID NO: 4

SEQUENCE LENGTH: 31 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAGCTTATTA CATTGCCGA AGAGCCCTCA G

SEQ ID NO: 5

SEQUENCE LENGTH: 25 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CGGTCATGAA GATCACCGCT GTCAT

SEQ ID NO: 6

SEQUENCE LENGTH: 27 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

GGGAAGCTTA GCTCTCAAAT TTGGCAG

EP 0 773 296 A1

SEQ ID NO: 7

SEQUENCE LENGTH: 185 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

Sequence

Met Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
1 5 10 15
Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
20 25 30
Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
35 45
Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
50 55 60
Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
65 70 75 80
Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
85 90 95
Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
100 105 110
Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
115 120 125
Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr
130 135 140
Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln
145 150 155 160
Asp Met Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln
165 170 175
Ser Ser Leu Arg Ala Leu Arg Gln Met
180 185

SEQ ID NO: 8

SEQUENCE LENGTH: 30 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAAGAATTCC CAGTACCCCC AACCTCTCA

SEQ ID NO: 9

SEQUENCE LENGTH: 31 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAGCTTATTA CATTTGCCGA AGAGCCCTCA G

SEQ ID NO: 10

SEQUENCE LENGTH: 162 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: protein

Sequence

Pro Val Pro Pro Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr

1 5 10 15

Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Glu Ser Ser Lys

20 25 30

EP 0 773 296 A1

Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu Lys
 35 45
 Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys
 50 55 60
 Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln
 65 70 75 80
 Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met Ser
 85 90 95
 Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp
 100 105 110
 Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys
 115 120 125
 Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile
 130 135 140
 Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg
 145 150 155 160
 Gln Met
 162

SEQ ID NO: 11

SEQUENCE LENGTH: 29 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CTTGGCGCCC CAGTACCCCC AACCTCTTC

SEQ ID NO: 12

SEQUENCE LENGTH: 10 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAAATGATTT AAAGGCTATA

SEQ ID NO: 13

SEQUENCE LENGTH: 20 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

TTGACTAGTT ATTAATAGTA

SEQ ID NO: 14

SEQUENCE LENGTH: 26 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CCAAGCGCTA ACTGAAACCA CACCAG

EP 0 773 296 A1

SEQ ID NO: 15

SEQUENCE LENGTH: 163 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

Sequence

Ala Pro Val Pro Pro Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg
1 5 10 15
Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Glu Ser Ser
20 25 30
Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu
35 45
Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val
50 55 60
Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu
65 70 75 80
Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met
85 90 95
Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu
100 105 110
Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr
115 120 125
Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu
130 135 140
Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu
145 150 155 160
Arg Gln Met
163

SEQ ID NO: 16

SEQUENCE LENGTH: 30 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: Peptide

Sequence

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala
 1 5 10 15
 Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser
 20 25 30

SEQ ID NO: 17

SEQUENCE LENGTH: 29 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CTTGCGGCC CAGTACCCCC AACCTCTTC

SEQ ID NO: 18

SEQUENCE LENGTH: 20 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAAATGATTT AAAGGCTATA

EP 0 773 296 A1

SEQ ID NO: 19

SEQUENCE LENGTH: 20 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA
Sequence

TTGACTAGTT ATTAATAGTA

SEQ ID NO: 20

SEQUENCE LENGTH: 29 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA
Sequence

CCAAGCGCTC TTGCTAACTG AAACCACAC

SEQ ID NO: 21

SEQUENCE LENGTH: 32 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

Sequence

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala

1

5

10

15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Lys Ser

20

25

30

32

SEQ ID NO: 22

SEQUENCE LENGTH: 35 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CCTCTTAAGA AGCGTCCAGT ACCCCCAACC TCTTC

SEQ ID NO: 23

SEQUENCE LENGTH: 20 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAAATGATT AAAGGCTATA

SEQ ID NO: 24

SEQUENCE LENGTH: 20 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

TTGACTAGTT ATTAATAGTA

EP 0 773 296 A1

SEQ ID NO: 25

SEQUENCE LENGTH: 29 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CTTCTTAAGG CTAAGTAAA CCACACCAG

SEQ ID NO: 26

SEQUENCE LENGTH: 34 amino acids

SEQUENCE TYPE: amino acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

Sequence

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala

1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Leu Lys

20 25 30

Lys Arg

34

SEQ ID NO: 27

SEQUENCE LENGTH: 35 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

TTTCTTAAGA AGCGTACATG TGAATTCGAG CTCGG

SEQ ID NO: 28

SEQUENCE LENGTH: 36 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

AAAAGATCTG ATATCGTCTT GTGACGTCAT TTTATT

SEQ ID NO: 29

SEQUENCE LENGTH: 75 base pairs

SEQUENCE TYPE: nucleic acid

STRANDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid synthetic DNA

Sequence

CTTAAGAAGC GTACATGTGA ATTCGAGCTC GGTACCCGGG GATCCTCTAG AGTCGACCTG 60
CAGGCATGCA AGCTT 75

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: ASAHI GLASS COMPANY LTD.
 (B) STREET: 1-2, Marunouchi 2-chome, Chiyoda-ku
 (C) CITY: TOKYO
 (E) COUNTRY: 100 JAPAN
 (F) POSTAL CODE (ZIP): none

(ii) TITLE OF INVENTION: SECRETION SIGNAL GENE AND EXPRESSION VECTOR
 CONTAINING IT

(iii) NUMBER OF SEQUENCES: 29

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 201 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met	Lys	Ile	Thr	Ala	Val	Ile	Ala	Leu	Leu	Phe	Ser	Leu	Ala	Ala	Ala	1	5	10	15
Ser	Pro	Ile	Pro	Val	Ala	Asp	Pro	Gly	Val	Val	Ser	Val	Ser	Lys	Ser	20	25	30	
Tyr	Ala	Asp	Phe	Leu	Arg	Val	Tyr	Gln	Ser	Trp	Asn	Thr	Phe	Ala	Asn	35	40	45	
Pro	Asp	Arg	Pro	Asn	Leu	Lys	Lys	Arg	Glu	Phe	Glu	Ala	Ala	Pro	Ala	50	55	60	
Lys	Thr	Tyr	Ala	Asp	Phe	Leu	Arg	Ala	Tyr	Gln	Ser	Trp	Asn	Thr	Phe	65	70	75	80
Val	Asn	Pro	Asp	Arg	Pro	Asn	Leu	Lys	Lys	Arg	Glu	Phe	Glu	Ala	Ala	85	90	95	
Pro	Glu	Lys	Ser	Tyr	Ala	Asp	Phe	Leu	Arg	Ala	Tyr	His	Ser	Trp	Asn	100	105	110	
Thr	Phe	Val	Asn	Pro	Asp	Arg	Pro	Asn	Leu	Lys	Lys	Arg	Glu	Phe	Glu	115	120	125	
Ala	Ala	Pro	Ala	Lys	Thr	Tyr	Ala	Asp	Phe	Leu	Arg	Ala	Tyr	Gln	Ser				

EP 0 773 296 A1

5 130 135 140

Trp Asn Thr Phe Val Asn Pro Asp Arg Pro Asn Leu Lys Lys Arg Thr
145 150 155 160

Glu Glu Asp Glu Glu Asn Glu Glu Glu Asp Glu Glu Tyr Tyr Arg Phe
 165 170 175

10 Leu Gln Phe Tyr Ile Met Thr Val Pro Glu Asn Ser Thr Ile Thr Asp
 180 185 190

Val Asn Ile Thr Ala Lys Phe Glu Ser
 195 200

(2) INFORMATION FOR SEQ ID NO: 2:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 59 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

25 Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala
 1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Lys Ser
 20 25 30

30 Tyr Ala Asp Phe Leu Arg Val Tyr Gln Ser Trp Asn Thr Phe Ala Asn
 35 40 45

Pro Asp Arg Pro Asn Leu Lys Lys Arg Glu Phe
 50 55

35 (2) INFORMATION FOR SEQ ID NO: 3:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
40 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GAATTCATGC CAGTACCCCC AGGAGAAGAT

30

(2) INFORMATION FOR SEQ ID NO: 4:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid

55

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
AAGCTTATTA CATTGCGGA AGAGCCCTCA G

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "syntheitc DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
CGGTCATGAA GATCACCGCT GTCAT

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
GGGAAGCTTA GCTCTCAAAT TTGGCAG

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 185 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His
 1 5 10 15
 Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr
 20 25 30
 Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser
 35 40 45
 Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn
 50 55 60
 Leu Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn
 65 70 75 80
 Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu
 85 90 95
 Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln
 100 105 110
 Ala Arg Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln
 115 120 125
 Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr
 130 135 140
 Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln
 145 150 155 160
 Asp Met Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln
 165 170 175
 Ser Ser Leu Arg Ala Leu Arg Gln Met
 180 185

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAAGAATTCC CAGTACCCCC AACCTCTTCA

30

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAGCTTATTA CATTGCCGA AGAGCCCTCA G

31

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 162 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Pro	Val	Pro	Pro	Thr	Ser	Ser	Glu	Arg	Ile	Asp	Lys	Gln	Ile	Arg	Tyr	1	5	10	15
Ile	Leu	Asp	Gly	Ile	Ser	Ala	Leu	Arg	Lys	Glu	Thr	Glu	Ser	Ser	Lys	20	25	30	
Glu	Ala	Leu	Ala	Glu	Asn	Asn	Leu	Asn	Leu	Pro	Lys	Met	Ala	Glu	Lys	35	40	45	
Asp	Gly	Cys	Phe	Gln	Ser	Gly	Phe	Asn	Glu	Glu	Thr	Cys	Leu	Val	Lys	50	55	60	
Ile	Ile	Thr	Gly	Leu	Leu	Glu	Phe	Glu	Val	Tyr	Leu	Glu	Tyr	Leu	Gln	65	70	75	80
Asn	Arg	Phe	Glu	Ser	Ser	Glu	Glu	Gln	Ala	Arg	Ala	Val	Gln	Met	Ser	85	90	95	
Thr	Lys	Val	Leu	Ile	Gln	Phe	Leu	Gln	Lys	Lys	Ala	Lys	Asn	Leu	Asp	100	105	110	
Ala	Ile	Thr	Thr	Pro	Asp	Pro	Thr	Thr	Asn	Ala	Ser	Leu	Leu	Thr	Lys	115	120	125	
Leu	Gln	Ala	Gln	Asn	Gln	Trp	Leu	Gln	Asp	Met	Thr	Thr	His	Leu	Ile	130	135	140	
Leu	Arg	Ser	Phe	Lys	Glu	Phe	Leu	Gln	Ser	Ser	Leu	Arg	Ala	Leu	Arg	145	150	155	160
Gln	Met																		

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 163 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ala Pro Val Pro Pro Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg
 1 5 10 15
 Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Glu Ser Ser
 20 25 30
 Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu
 35 40 45
 Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val
 50 55 60
 Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu
 65 70 75 80
 Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met
 85 90 95
 Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu
 100 105 110
 Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr
 115 120 125
 Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu
 130 135 140
 Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu
 145 150 155 160
 Arg Gln Met

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala

EP 0 773 296 A1

1 5 10 15
 Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CTTGGCGCCC CAGTACCCCC AACCTCTTC 29

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AAAATGATTT AAAGGCTATA 20

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TTGACTAGTT ATTAATAGTA 20

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CCAAGCGCTC TTGCTAACTG AAACCACAC

29

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala
1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Lys Ser
20 25 30

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CCTCTTAAGA AGCGTCCAGT ACCCCCAACC TCTTC

35

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

EP 0 773 296 A1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AAAATGATTT AAAGGCTATA

20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TTGACTAGTT ATTAATAGTA

20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TTCTTAAGG CTAAGTGAAG CCACACCAG

29

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Lys Ile Thr Ala Val Ile Ala Leu Leu Phe Ser Leu Ala Ala Ala
1 5 10 15

Ser Pro Ile Pro Val Ala Asp Pro Gly Val Val Ser Val Ser Leu Lys
20 25 30

Lys Arg

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TTTCTTAAGA AGCGTACATG TGAATTCGAG CTCGG

35

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

AAAAGATCTG ATATCGTCTT GTGACGTCAT TTTATT

36

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CTTAAGAAGC GTACATGTGA ATTCGAGCTC GGTACCCGGG GATCCTCTAG AGTCGACCTG

60

CAGGCATGCA AGCTT

75

Claims

1. A secretion signal gene having a base sequence encoding a polypeptide functional as a secretion signal in Schizosaccharomyces pombe.

EP 0 773 296 A1

2. The secretion signal gene according to Claim 1, wherein the polypeptide is a secretion signal of the P-factor precursor produced by S.pombe or a polypeptide containing the amino acid sequence of the secretion signal in which addition, deletion or substitution of at least one amino acid residue may be made.

3. A secretion signal gene having a base sequence encoding a polypeptide having an amino acid sequence from the 1st amino acid to the 16 - 160th amino acid of SEQ ID NO: 1 in which addition, deletion or substitution of at least one amino acid residue may have been made.

4. The secretion signal gene according to Claim 3, wherein the polypeptide has an amino acid sequence from the 1st amino acid to the 22±6th amino acid, from the 1st amino acid to the 31±6th amino acid, or from the 1st amino acid to the 57±6th amino acid of SEQ ID NO: 1.

5. The secretion signal gene according to Claim 3, wherein the polypeptide has at least one additional amino acid residue at the carboxy terminus so as to have a carboxy-terminal amino acid sequence of [-Lys-Lys-Arg].

6. A multicloning vector for construction of an expression vector for expression of a foreign protein in a eucaryotic host cell, which has the secretion signal gene according to Claim 1 or 3 upstream from a foreign protein structural gene introduction site so that the secretion signal gene can be directly ligated with a foreign protein structural gene to be introduced.

7. An expression vector to be expressed in a eucaryotic host cell, which has a structural gene of a protein combination of a secretion signal and a foreign protein bonded together in this order from the amino terminus wherein the gene of the secretion signal is the secretion signal gene according to Claim 1 or 3.

8. The expression vector according to Claim 7, which has an animal virus-derived promoter region governing the structural gene.

9. The expression vector according to Claim 7, which has a neomycine resistance gene region and a second animal virus-derived promoter governing the neomycine resistance gene region.

10. The expression vector according to Claim 7, wherein the eucaryotic host cell is Schizosaccharomyces pombe.

11. A transformant of a eucaryotic host cell, which carries a recombinant DNA containing a structural gene of a protein combination of the secretion signal gene according to Claim 1 or 3 and a foreign protein bonded together in this order from the amino terminus, or the expression vector according to Claim 7.

12. The transformant according to Claim 11, wherein the eucaryotic host cell is Schizosaccharomyces pombe.

13. A method of producing a foreign protein, which comprises culturing the transformant of Claim 11 so that the foreign protein is produced and accumulated in the culture, and collecting it.

FIGURE 1

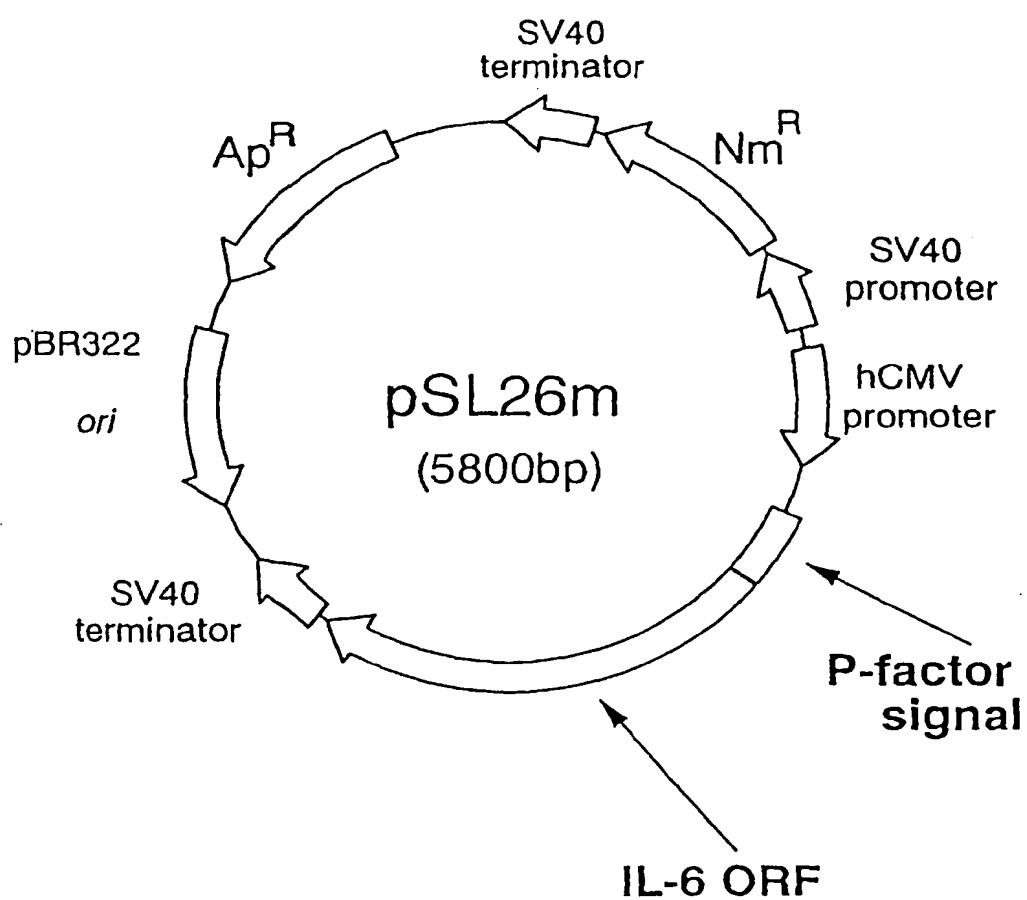


FIGURE 2

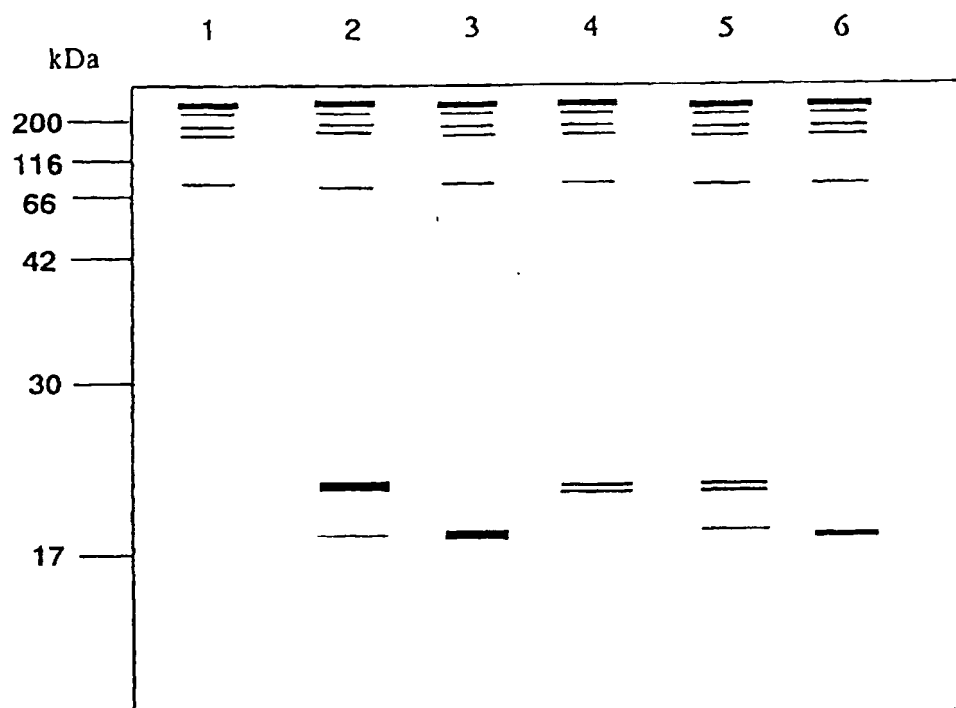


FIGURE 3

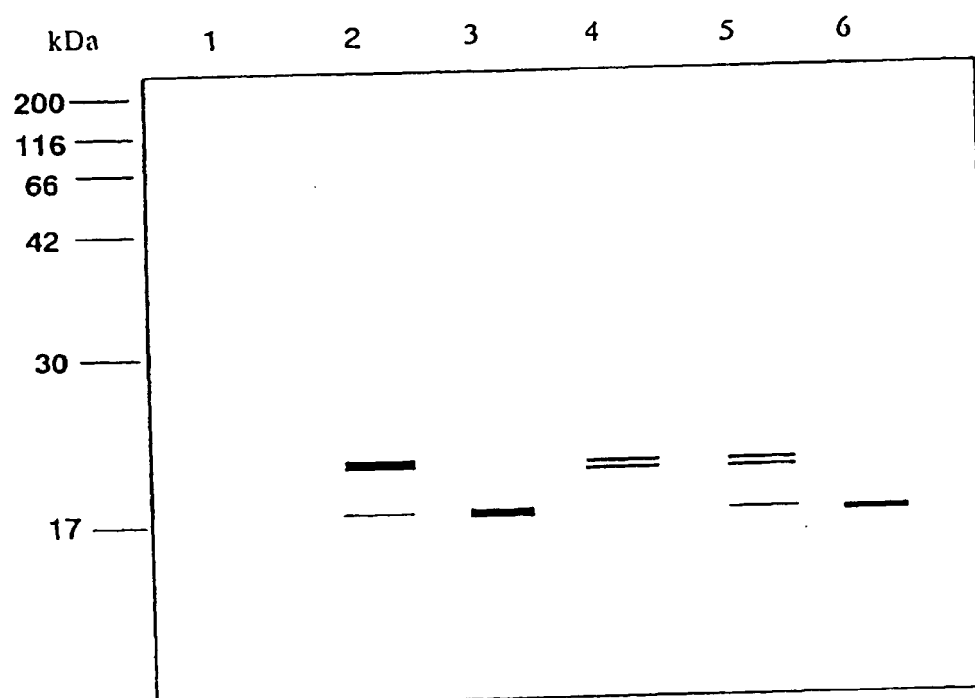


FIGURE 4

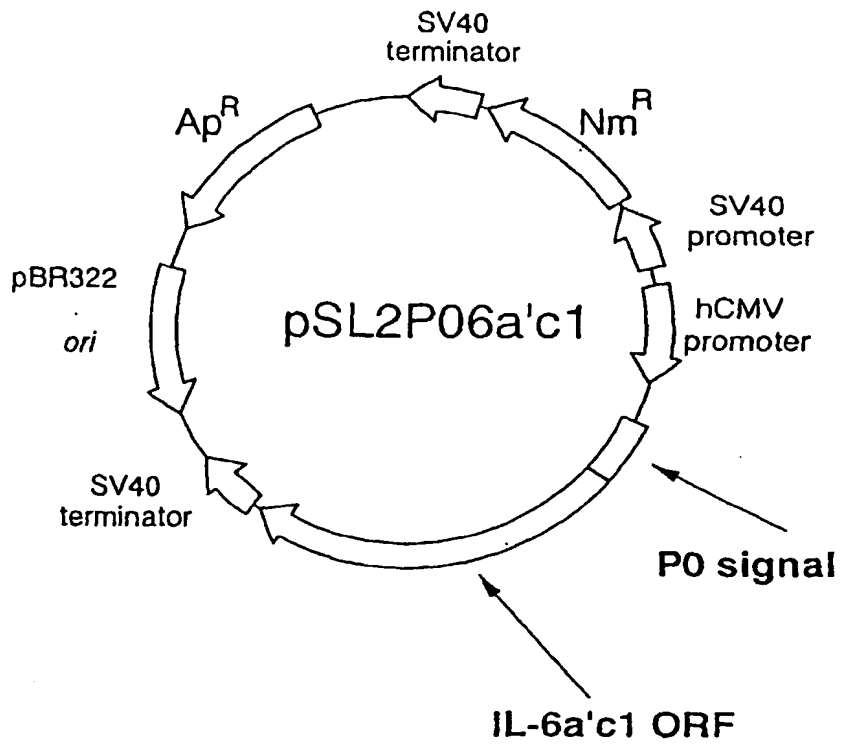


FIGURE 5

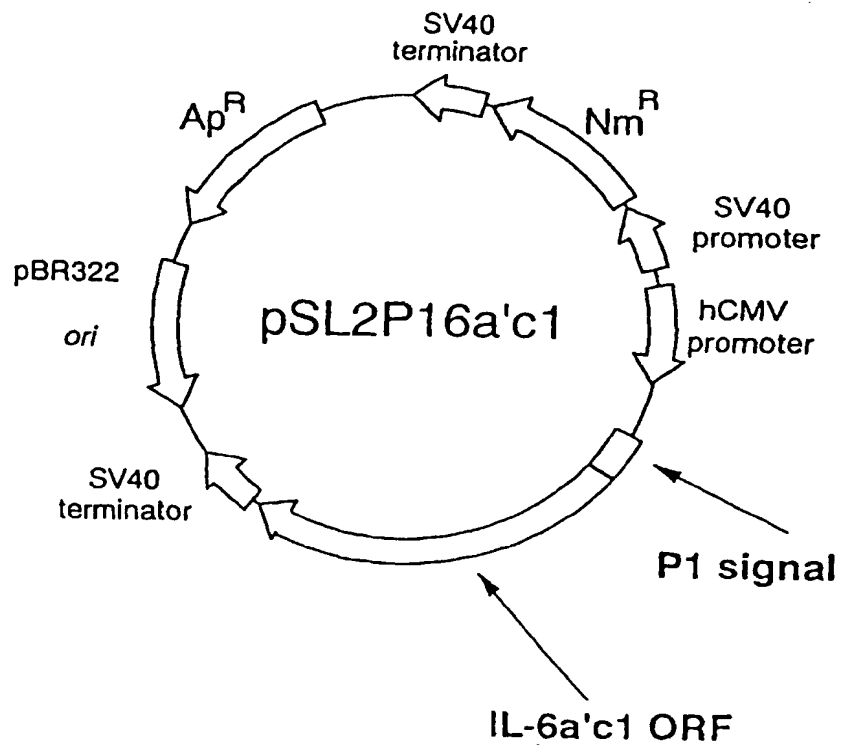


FIGURE 6

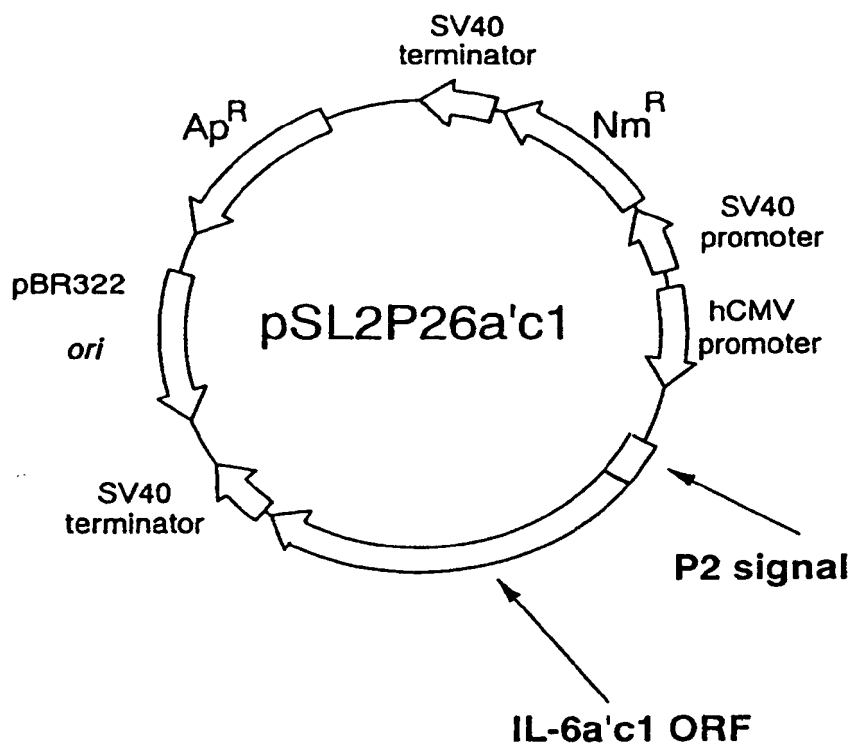


FIGURE 7

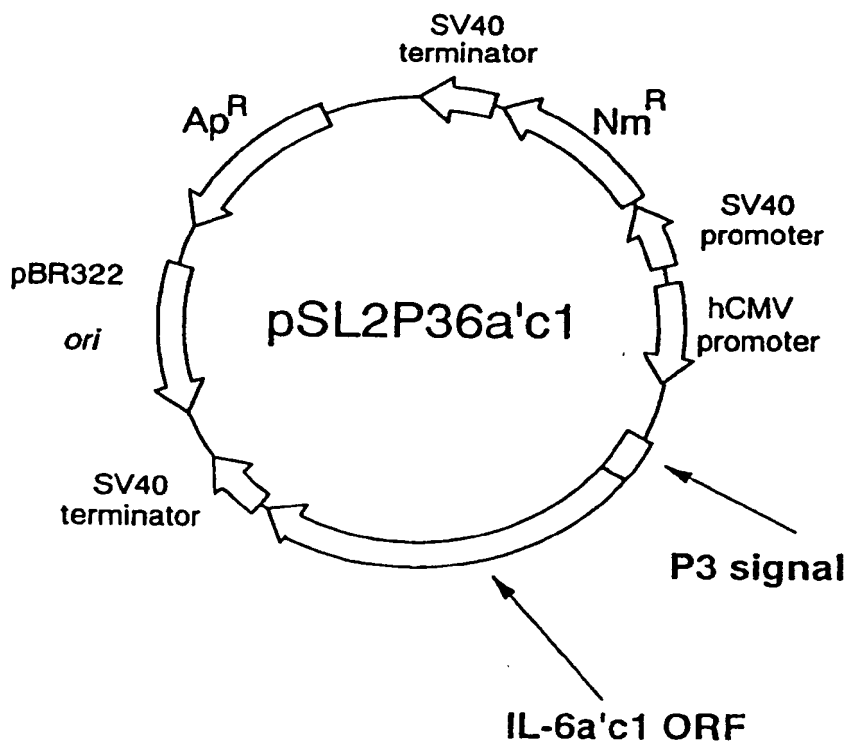
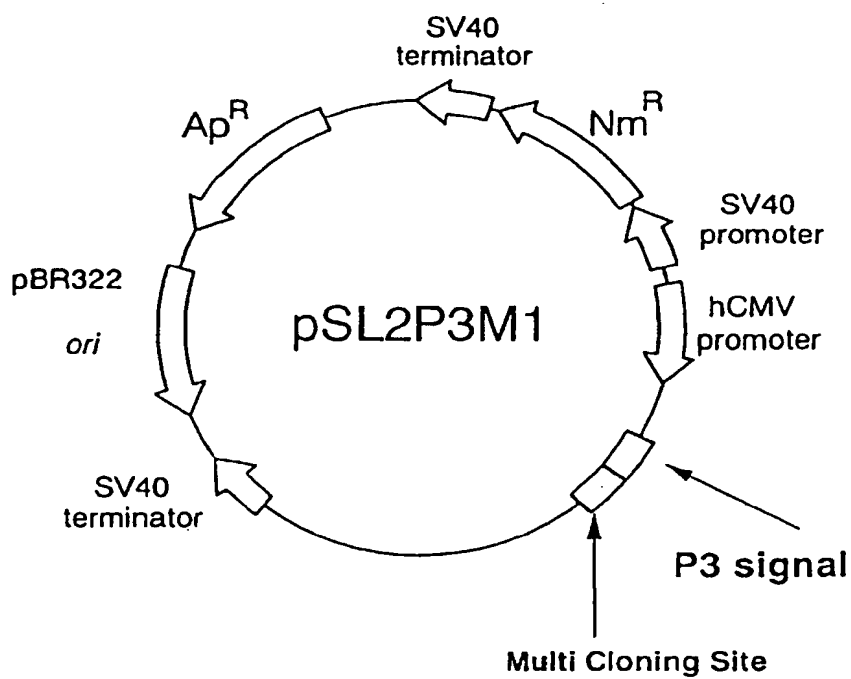


FIGURE 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP96/00198

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl⁶ C12N15/81, C12N15/31, C12N1/19, C12P21/02// (C12N1/19, C12R1:645)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl⁶ C12N15/81, C12N15/31, C12N1/19, C12P21/02// (C12N1/19, C12R1:645)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREVIEWS, CAS ONLINE, WPI, WPI/L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Hidetake Higashida et al. "A new method for producing useful proteins by means of Fission Yeast <i>S. pombe</i> ." BIO INDUSTRY (1994) Vol. 11, No. 11, p. 678-684	1 - 13
Y	Imai, Y. et al. "The fission yeast mating pheromone P-factor: its molecular structure, gene structure, and ability to induce gene expression and G1 arrest in the mating partner", Genes Dev. (1994) Vol. 8, No. 3, p. 328-338	1 - 13
Y	JP, 6-327481, A (Asahi Glass Co., Ltd.), November 29, 1994 (29. 11. 94) (Family: none)	1 - 13
X	Tokunaga, M. et al. "Secretion of Mouse α -Amylase from Fission Yeast <i>Schizosaccharomyces pombe</i> : Presence of Chymostatin-sensitive Protease Activity in Culture Medium", Yeast (1994) Vol. 9, No. 4, p. 379-387	1
X	Broeker, M. et al. "Expression of human	1

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

Date of the actual completion of the international search

April 18, 1996 (18. 04. 96)

Date of mailing of the international search report

April 30, 1996 (30. 04. 96)

Name and mailing address of the ISA/

Japanese Patent Office

Facsimile No.

Authorized officer

Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP96/00198

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	antithrombin III in <i>Saccharomyces cerevisiae</i> and <i>Schizosaccharomyces pombe</i> ", <i>Biochim. Biophys. Acta</i> (1987) Vol. 908, No. 3, p. 203-213	
X	JP, 4-63596, A (Takeda Chemcial Industries, Ltd.), February 28, 1992 (28. 02. 92) (Family: none)	1
X	JP, 3-175976, A (Takeda Chemical Industries, Ltd.), July 31, 1991 (31. 07. 91) (Family: none)	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

BEST AVAILABLE COPY